most effective cord blood processing method results in the highest total nucleated cell (TNC) count recovery with RBC volume of <20 ml. In order to determine the most efficient automated and manual processes, we compared units RBC-depleted by elutriation (Sepax) with units that were RBC-depleted manually. Sepax processing was performed with final processing volumes of 45 ml (n = 1001), 35 ml (n = 18) or 25 ml (n = 9); manual processing was performed by removing the equivalent of 0-40% of the starting volume from the RBC pellet (40% n = 17; 30% n = 9); 20% n = 14; 10% n = 6 and 0% n = 18). The TNC recoveries of units processed by Sepax were equivalent at 45 ml (88%), 35 ml (87%), and 25 ml (88%). Units processed with manual RBC depletion resulted in decreased TNC recoveries with greater RBC depletion (90% recovery at 0% reduction vs. 81% TNC recovery at 40% reduction). The mean final RBC volumes were significantly lower in the units processed by Sepax (5.9 ml at 25 ml; 9.5 ml at 35 ml; 14.6 ml at 45 ml) compared to the units that were manually RBC-depleted (17 ml at 40% reduction vs. 27 ml at 0% reduction). We conclude that RBC depletion to <20 ml can be routinely achieved with any Sepax method or by manual depletion of 40% of the starting volume from the RBC pellet.

Table 1. Imaging Characteristics of Experimental Animals and Histologic Correlations

Experimental Rat Subsets	FLT studies performed	FDG studies performed	Histologic Correlation
Normal/Baseline rat studies	n = 10	n = 6	Normal cellular marrow
24-48 hrs post 950 cGy TBI	n = 6	n = 4	Marrow damage hypocellularity
Day 7 post 950 cGy TBI	n = 4	not performed	Aplastic marrow
Day 6-7 post 950 cGy TBI (4-5 days post transplantation)	n = 4	n = 4	Focal areas of cellular regeneration
Day 10 post 950 cGy TBI (6 days post transplantation	n = 4	n = 2	Cellular marrow
Day 6-7 post 500 cGy TBI (no transplantation)	n = 6	not performed	Moderate hypocellularity

HEMATOPOIESIS/MESENCHYMAL CELLS

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THE USE OF F-18 THYMIDINE IMAGING FOR EVALUATION OF THE BONE MARROW COMPARTMENT FOLLOWING TRANSPLANTATION

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Evaluation of engraftment following bone marrow transplantation is based on measurements of marrow growth including peripheral blood counts and bone marrow biopsies done weeks following transplant. Early prediction of marrow function becomes increasingly necessary as cord blood use increases, in evaluation of intentional or unintentional radiation exposure, for evaluation of minimal residual disease, and to maximize treatment efficacy in hematologic malignancies. F-18 Flourothymidine (FLT)/PET imaging is being evaluated clinically for tumor detection and staging. Unlike the more commonly used FDG/PET, FLT is tied to proliferative activity and cellular growth by thymidine's inclusion into DNA, and is therefore less hampered by inflammatory background. We describe the use of FLT in two groups, 1) a rat model of ablative and non-myeloablative transplantation, and 2) normal and lymphoma patients undergoing treatment.

In our rat model, we evaluated if imaging could provide early evidence of hematopoietic recovery following radiation and transplantation. Rats received myeloablative, non-myeloablative, and sham transplantation with imaging at baseline, 24-48 hours after transplantation and 6 days post-transplantation. As seen in Table 1, analysis confirms that FLT predicts engraftment as early as 24-48 hours following transplantation, FLT also can predict lack of engraftment and radiation effect in sham transplantations who did not receive stem cell infusion. Figure 1 illustrates FLT and FDG imaging performed in a normal, post radiation and post transplant rat, confirming superiority of FLT in evaluating marrow function.

In our human model, we evaluated if imaging could be used to provide useful information about chemotherapeutic and radiation effects on marrow. We imaged 6 patients with intracranial malignancy with normal bone marrow as controls. In addition, we imaged 3 lymphoma patients prior to treatment, at day 9 post-chemotherapy, and following six cycles of chemotherapy. Figure 2 illustrates differences seen in FLT in a patient with normal marrow and a patient with known marrow involvement. Our human and animal model confirm that FLT can be used as a marker of cellular proliferation in the bone marrow. We propose that this tool could be used for early evaluation of engraftment, success of chemotherapy, prediction of radiation injury, and evaluation of minimal residual disease.

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SUICIDE CASPASE 9 GENE FOR IMPROVED SAFETY OF THERAPIES USING MESENCHYMAL STROMAL CELLS

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Although MSC have been administered to hundreds of patients so far with minimal reported side effects, little is known about their long term effects. Recent reports of osteosarcoma formation in culture and interference with major organ function due to ectopic ossification raise safety concerns. In light of these, we sought to develop a system allowing control over the growth and survival of MSCs administered therapeutically, using an inducible caspase-9 (iCasp9) protein that can be activated by a specific chemical inducer of dimerization (CID) leading to cell death.

MSCs from healthy donors were retrovirally transduced with iCasp9 linked to a truncated form of CD19, allowing selection of transduced cells: 47 ± 6% of the cells were iCasp9$^+$, a percentage stable over more than two weeks in culture, suggesting no deleterious or growth advantageous effects of the construct on MSCs. iCasp9$^+$ cells were easily immunomagnetically selected. Phenotype (>99% CD73$^+$CD90$^+$CD105$^+$, and negative for hematopoietic markers) and differential potential of iCasp9$^+$ cells was identical to that of untransduced cells, proving the genetic manipulation of MSCs did not modify their basic characteristics. Non-transduced MSCs had a spontaneous rate of apoptosis in culture ~18% (±7%), similar to iCasp9$^+$ cells at baseline (15 ± 6%, $P = 0.47$). Addition of CID to cultures after transduction resulted in apoptotic death of >90% of iCasp9$^+$ cells (93 ± 1%, $P < 0.0001$), while iCasp9$^-$ cells retained an apoptosis index similar to that of non-transduced controls (20 ± 7%, $P = 0.99$ and $P = 0.69$ vs. non-transduced controls with or without CID respectively), demonstrating that iCasp9$^+$ MSCs can be selectively killed. Addition of CID to cultures of differentiated MSCs resulted in widespread apopotosis as assessed by TUNEL assay. Furthermore, using an in vivo imaging system, we saw that iCasp9$^+$ MSCs injected subcutaneously in SCID mice were selectively eliminated after administration of CID, without toxic effects.

In summary, MSCs are easily transduced with our iCasp9 system, the transduced cells can be selected with clinical grade procedures and maintain their basic physiology, and their differentiated progeny can be selectively eliminated by exposure to a CID, analogues of which have been safely tested in a phase I study. This directed MSC killing provides a necessary safety mechanism for therapies using progenitor cells. This approach will become of increasing value as clinical applications for MSCs develop further.